

FINAL REPORT

Test Facility Study No. 511872

IN VITRO SKIN IRRITATION TEST WITH MLA-3202 USING A HUMAN SKIN MODEL

SPONSOR:

Chemtura Corporation
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TEST FACILITY:

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15 June 2016

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1. STATEMENT OF GLP COMPLIANCE

WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands

All phases of this study performed by the test facility were conducted in compliance with the following GLP regulations:

- OECD Principles of Good Laboratory Practice concerning Mutual Acceptance of Data in the Assessment of Chemicals, 26 November 1997 (C(97) 186 Final);
- EC Council Directive 2004 (2004/10/EC, February 11, 2004, Official Journal of February 20, 2004).

Except for the following:

- The quality environment in which the characterisation of the test item was performed was not known.

The data generated and reported are considered to be valid.

WIL Research Europe B.V.

Signature: 

Name: I.M.J. Eurlings, PhD.

Title: Study Director

Date: 15 January

2. TEST FACILITY QUALITY ASSURANCE STATEMENT

WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands.

Study title: *In vitro* skin irritation test with MLA-3202 using a human skin model.

This report was inspected by the WIL Research Europe Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s).

The reported method and procedures were found to describe those used and the report reflects the raw data.

During the on-site process inspections, procedures applicable to this type of study were inspected.

The dates of Quality Assurance inspections are given below.

Project	511872			
Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Study Plan Report	04-May-2016 13-Jun-2016	04-May-2016 13-Jun-2016	04-May-2016 14-Jun-2016
Process	Genetic and In Vitro Toxicology Test Substance Handling Exposure Observations/Measurements Specimen Handling	22-Mar-2016	31-Mar-2016	04-Apr-2016
	Test Substance Receipt Test Substance Handling	09-May-2016	20-May-2016	24-May-2016

The facility inspection program is conducted in accordance with Standard Operating Procedure.

The review of the final report was completed on the date of signing this QA statement.

WIL Research Europe B.V.

Signature: 

Name: C. Mitchell B.Sc., FRQA
Head of Quality Assurance

Date: 15 Jun 2016

3. SUMMARY

The objective of this study was to evaluate the potential of MLA-3202 to induce skin irritation using a human three dimensional epidermal model (EPISKIN Small Model (EPISKIN-SMTM))). The possible skin irritation potential of MLA-3202 was tested through topical application for 15 minutes.

The study procedures described in this report were designed to be compatible with the most recent OECD and EC guidelines.

Batch RC-1045 of MLA-3202 was a clear amber-red liquid. MLA-3202 was applied undiluted (25 µl), directly on top of the skin tissue for 15 ± 0.5 minutes. After a 42 hour post-incubation period, determination of the cytotoxic (irritancy) effect was performed. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT at the end of the treatment.

Skin irritation is expressed as the remaining cell viability after exposure to the test item. The relative mean tissue viability obtained after 15 ± 0.5 minutes treatment with the test item compared to the negative control tissues was 94%. Since the mean relative tissue viability for MLA-3202 was above 50% after 15 ± 0.5 minutes treatment MLA-3202 is considered to be non-irritant.

The positive control had a mean cell viability of 12% after 15 ± 0.5 minutes exposure. The absolute mean OD₅₇₀ (optical density at 570 nm) of the negative control tissues was within the laboratory historical control data range. The standard deviation value of the percentage viability of three tissues treated identically was less than 15%, indicating that the test system functioned properly.

Finally, it is concluded that this test is valid and that MLA-3202 is non-irritant in the *in vitro* skin irritation test under the experimental conditions described in this report.

4. INTRODUCTION

Experimental starting date : 10 May 2016
Experimental completion date : 17 May 2016

4.1. Purpose

MLA-3202 has been tested previously in a Skin corrosion test using EpiDerm as a skin model and was found not corrosive (project 511871). The objective of this study was to evaluate MLA-3202 for its ability to induce skin irritation using Episkin as a skin model. EpiDerm and Episkin are recommended for testing skin corrosion and skin irritation, respectively. For this purpose MLA-3202 was topically applied on a human three dimensional epidermal model.

Background of the test system

The test is based on the experience that irritant chemicals show cytotoxic effects following short term exposure to the stratum corneum of the epidermis. The test is designed to predict and classify the skin irritation potential of a test item by assessment of its effect on a three dimensional human epidermis model (1-10).

The test consists of topical application of the test item on the skin tissue for 15 minutes. After exposure the skin tissue is thoroughly rinsed to remove the test item and transferred to fresh medium. After a 42 hour incubation period, determination of the cytotoxic (irritancy) effect is performed.

Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at the end of the treatment.

4.2. Guidelines

The study procedures described in this report are in compliance with the following guidelines:

- Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Guideline no. 439: *In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method* (adopted 28 July 2015).
- European Community (EC). Commission regulation (EC) No. 440/2008, Part B: Methods for the Determination of Toxicity and other health effects, Guideline B.46 "*In Vitro Skin Irritation: Reconstructed Human Epidermis Model Test*". Official Journal of the European Union No. L142; Amended by EC No. 640/2012 OJ No. L193, 20 July 2012.

4.3. Retention of records and materials

Records and material pertaining to the study, which include study plan and amendments, raw data, specimens, except perishable specimens, and the final report will be retained in the archives of the test facility for a minimum of 5 years after the finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. The test facility will retain information concerning decisions made.

Perishable specimens (e.g. requiring refrigeration or freezing) will be discarded following evaluation in the study without further notice to the study sponsor.

A sample of the test item will be retained until expiry date or applicable retest date. After this period the sample(s) will be destroyed.

4.4. Responsible personnel

4.4.1. Test facility

Study Director I.M.J. Eurlings, PhD.

4.4.2. Sponsor Representative

Study Monitor Audrey Batoon, Ph.D.

5. MATERIALS AND METHODS

5.1. Test item

5.1.1. Test item information

Test item number	207258/A
Identification	MLA-3202
Appearance	Clear amber-red liquid
Batch	RC-1045
Purity/Composition	UVCB
Test item storage	At room temperature
Stable under storage conditions until	17 February 2019 (expiry date)

5.1.2. Study specific test item information

Purity/composition correction factor	No correction factor required
Test item handling	No specific handling conditions required
Stability at higher temperatures	Stable
Chemical name (IUPAC), synonym or trade name	Amides, tallow, N,N-bis(2-hydroxypropyl)
CAS Number	1454803-04-3

5.2. Reference items

5.2.1. Negative control:

Phosphate buffered saline (PBS, Merck KGaA, Darmstadt, Germany).

5.2.2. Positive control:

5% (aq) Sodium dodecyl sulphate (SDS, Sigma, Zwijndrecht, The Netherlands) [CAS Number 151-21-3] in PBS.

5.3. Test item preparation

The liquid test item was applied undiluted (25 µl) directly on top of the tissue.

5.4. Test system

Test system

EPISKIN Small Model™ (EPISKIN-SM™, 0.38 cm², Batch no.: 16-EKIN-019, [APPENDIX 4](#)). This model is a three-dimensional human epidermis model, which consists of adult human-derived epidermal keratinocytes which have been seeded on a dermal substitute consisting of a collagen type I matrix coated with type IV collagen. The keratinocytes were cultured for 13 days, which results in a highly differentiated and stratified epidermis model comprising the main basal, supra basal, spinous and granular layers and a functional stratum corneum.

Rationale

In the interest of sound science and animal welfare, a sequential testing strategy is recommended to minimise the need of *in vivo* testing. One of the validated *in vitro* skin irritation tests is the EPISKIN test, which is recommended in international guidelines (e.g. OECD and EC).

Source

SkinEthic Laboratories, Lyon, France.

5.5. Preparation and preincubation

Tissues

On the day of receipt the tissues were transferred to 12-well plates and preincubated with prewarmed Maintenance Medium for approximately 24 hours at 37°C ([Figure 1](#)). Maintenance medium and Assay medium were supplied by Skinethic Laboratories, Lyon, France.

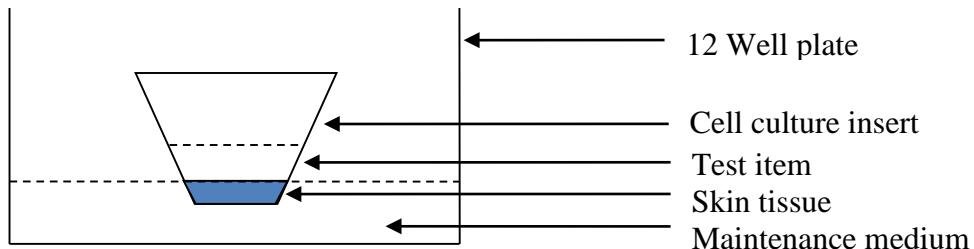


Figure 1 A diagram of the application.

MTT medium

MTT concentrate (Sigma Aldrich, Zwijndrecht, The Netherlands; 3 mg/ml in PBS) diluted (10x) in Assay medium (final concentration 0.3 mg/ml).

Environmental conditions

All incubations, with the exception of the test item incubation of 15 minutes at room temperature, were carried out in a controlled environment, in which optimal conditions were a humid atmosphere of 80 - 100% (actual range 67 - 89%), containing 5.0 ± 0.5% CO₂ in air in the

dark at $37.0 \pm 1.0^\circ\text{C}$ (actual range $34.9 - 37.0^\circ\text{C}$). Temperature and humidity were continuously monitored throughout the experiment. The CO₂ percentage was monitored once on each working day. Temporary deviations from the temperature, humidity and CO₂ percentage may occur due to opening and closing of the incubator door. Based on laboratory historical data these deviations are considered not to affect the study integrity.

5.6. Study design

5.6.1. Test for the interference of the test item with the MTT endpoint

A test item may interfere with the MTT endpoint if it is coloured and/or it is able to directly reduce MTT. The cell viability measurement is affected only if the test item is present on the tissues when the MTT viability test is performed.

MLA-3202 was checked for possible direct MTT reduction and colour interference in the Skin corrosion test using EpiDerm as a skin model (project 511871). Because solutions did not turn blue / purple and a blue / purple precipitate was not observed it was concluded that MLA-3202 did not interfere with the MTT endpoint.

5.6.2. Application/Treatment of the test item

The test was performed on a total of 3 tissues per test item together with negative and positive controls. Twenty five µl of the undiluted test item was added into 12-well plates on top of the skin tissues. Three tissues were treated with 25 µl PBS (negative control) and 3 tissues with 25 µl 5% SDS (positive control) respectively. The positive control was re-spread after 7 minutes contact time. After the exposure period of 15 ± 0.5 minutes at room temperature, the tissues were washed with phosphate buffered saline to remove residual test item. After rinsing, the cell culture inserts were each dried carefully and moved to a new well on 2 ml pre-warmed maintenance medium until all tissues were dosed and rinsed. Subsequently the skin tissues were incubated for 42 hours at 37°C .

5.6.3. Cell viability measurement

After incubation, cell culture inserts were dried carefully to remove excess medium and were transferred into a 12-wells plate pre-filled with 2 ml MTT-solution (0.3 mg/ml in PBS). The tissues were incubated for 3 h at 37°C . After incubation the tissues were placed on blotting paper to dry the tissues. Total biopsy was made by using a biopsy punch. Epidermis was separated from the collagen matrix and both parts were placed in pre-labeled microtubes and extracted with 500 µl isopropanol (Merck, Darmstadt, Germany). Tubes were stored refrigerated and protected from light for approximately 71 hours. The amount of extracted formazan was determined spectrophotometrically at 570 nm in duplicate with the TECAN Infinite® M200 Pro Plate Reader.

Cell viability was calculated for each tissue as a percentage of the mean of the negative control tissues. Skin irritation potential of the test item was classified according to remaining cell viability following exposure of the test item.

5.7. Interpretation

5.7.1. Acceptability of the assay

The *in vitro* skin irritation test is considered acceptable if it meets the following criteria:

- a) The absolute mean OD₅₇₀ (optical density at 570 nm) of the three tissues of the negative control should reasonably be within the laboratory historical control data range and the Standard Deviation value (SD) of the % viability should be ≤18.
- b) The mean relative tissue viability of the positive control should be ≤50% relative to the negative control and the Standard Deviation value (SD) of the % viability should be ≤18.
- c) The SD calculated from individual % tissue viabilities of the three identically treated replicates should be ≤18.

5.7.2. Data evaluation and statistical procedures

A test item is considered irritant in the skin irritation test if:

The relative mean tissue viability of three individual tissues after 15 minutes of exposure to the test item and 42 hours of post incubation is ≤ 50% of the mean viability of the negative controls.

A test item is considered non-irritant in the *in vitro* skin irritation test if:

The relative mean tissue viability of three individual tissues after 15 minutes of exposure to the test item and 42 hours of post incubation is > 50% of the mean viability of the negative controls.

Table 1 Data interpretation of test items

Relative mean viability of 3 individual tissues after 15 minutes of exposure and 42 hours of post incubation	Prediction to be considered
≤ 50% of the mean viability of the negative controls	Category 1 or Category 2 (additional information on corrosion needed)
> 50% of the mean viability of the negative controls	No category

5.8. List of deviations

5.8.1. List of study plan deviations

There were no deviations from the study plan.

5.8.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

6. ELECTRONIC SYSTEMS FOR DATA ACQUISITION

The following electronic systems were used for data acquisition:

- REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA): Temperature and humidity.
- Magellan Tracker 7.0 (TECAN, Austria) for optical density measurement.

7. RESULTS

The mean absorption at 570 nm measured after treatment with MLA-3202 and controls are presented in [APPENDIX 1, Table 2](#). The individual OD₅₇₀ measurements are presented in [APPENDIX 2](#).

[Table 3](#) shows the mean tissue viability obtained after 15 ± 0.5 minutes treatment with the test item compared to the negative control tissues. Skin irritation is expressed as the remaining cell viability after exposure to the test item. The relative mean tissue viability obtained after 15 ± 0.5 minutes treatment with MLA-3202 compared to the negative control tissues was 94%. Since the mean relative tissue viability for MLA-3202 was above 50% MLA-3202 is considered to be non-irritant.

The positive control had a mean cell viability after 15 ± 0.5 minutes exposure of 12%. The absolute mean OD₅₇₀ of the negative control tissues was within the laboratory historical control data range (See [APPENDIX 3](#)). The standard deviation value of the percentage viability of three tissues treated identically was less than 15%, indicating that the test system functioned properly.

8. CONCLUSION

Finally, it is concluded that this test is valid and that MLA-3202 is non-irritant in the *in vitro* skin irritation test under the experimental conditions described in this report and should not be classified according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) of the United Nations.

9. REFERENCES

1. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65: 55-63.
2. Ponec, M., Boelsma, E., Weerheim, A., Mulder, A., Bouwstra, J., Mommaas, M. (2000). Lipid and ultrastructural characterization of reconstructed skin models. *International Journal of Pharmaceutics* 203: 211 - 225.
3. Fentem, J.H., Briggs, D., Chesné, C., Elliott, G. R., Harbell, J.W., Heylings, J.R., Portes, P., Roguet, R., van de Sandt, J.J.M., Botham, P.A. (2001). A prevalidation study on *in vitro* tests for acute skin irritation results and evaluation by the management team. *Toxicology in vitro* 15: 57-93.
4. Zuang, V., Balls, M., Botham, P.A., Coquette, A., Corsini, E., Curren, R.D., Elliott, G.R., Fentem, J.H., Heylings, J.R., Liebsch, M., Medina, J., Roguet, R., Sandt van de, J.J.M., Wiemann, C., Worth, A.P. (2002). Follow-up to the ECVAM Prevalidation Study on *In Vitro* Tests for Acute Skin Irritation. *ATLA Alternatives to Laboratory animals* 30: 109-129.
5. Kandárová, H., Liebsch, M., Genschow, E., Gerner I., Traue, D., Slawik, B., Spielmann, H. (2004). Optimisation of the EpiDerm test protocol for the upcoming ECVAM validation study on *in vitro* skin irritation tests *ALTEX-Alternativen zu tierexperimenten* 21: 107-114.

6. Kandárová, H., Liebsch, M., Gerner, I., Schmidt, E., Genschow, E., Traue, D., Spielmann, H. (2005). The EpiDerm test protocol for the upcoming ECVAM validation study on in vitro skin irritation tests – an assessment of the performance of the optimised test. ATLA Alternatives to Laboratory animals 33: 351-367.
7. Cotovio, J., Grandidier, M-H., Portes, P., Roguet, R., Rubinstenn, G. (2005). The In Vitro Acute Skin Irritation of Chemicals: Optimisation of the EPISKIN Prediction Model within the Framework of the ECVAM Validation Process. ATLA Alternatives to Laboratory animals 33: 329-349.
8. Zuang, V., Alonso, M.A., Botham, P. A., Eskes, C., Fentem, J., Liebsch, M., van de Sandt, J.J.M. (2005). Skin irritation and corrosion. ATLA Alternatives to Laboratory animals 33: suppl. 1: 35-46.
9. Spielmann, H., Hoffmann, S., Liebsch, M., Botham, P., Fentem, J., Eskes, C., Roguet, R., Cotovió, J., Cole, T., Worth, A., Heylings, J., Jones, P., Robles, C., Kandárová, H., Gamer, A., Remmele, M., Curren, R., Raabe, H., Cockshott, A., Gerner, I., Zuang, V. (2007). The ECVAM International Validation Study on In Vitro Tests for Acute Skin Irritation: Report on the Validity of the EPISKIN and EpiDerm Assays and on the Skin Integrity Function Test. ATLA Alternatives to Laboratory animals 35: 559-601.
10. Eskes, C., Cole, T., Hoffmann, S., Worth, A., Cockshott, A., Gerner, I., Zuang, V (2007). ECVAM International Validation Study on In Vitro Tests for Acute Skin Irritation: Selection of Test Chemicals. ATLA Alternatives to Laboratory animals 35: 603-619.

APPENDIX 1
Tables**Table 2 Mean absorption in the *in vitro* skin irritation test with MLA-3202**

	A (OD ₅₇₀)	B (OD ₅₇₀)	C (OD ₅₇₀)	Mean (OD ₅₇₀)	SD
Negative control	0.755	0.924	0.968	0.882	± 0.112
MLA-3202	0.974	0.745	0.762	0.827	± 0.128
Positive control	0.115	0.136	0.069	0.107	± 0.035

OD = optical density

SD = Standard deviation

Triplicate exposures are indicated by A, B and C.

In this table the values are corrected for background absorption (0.041). Isopropanol was used to measure the background absorption.

Table 3 Mean tissue viability in the *in vitro* skin irritation test with MLA-3202

	Mean tissue viability (percentage of control)
Negative control	100
MLA-3202	94
Positive control	12

APPENDIX 2
Individual OD measurements at 570 nm

	A (OD ₅₇₀)	B (OD ₅₇₀)	C (OD ₅₇₀)
Negative control			
OD ₅₇₀ measurement 1	0.8261	0.9820	1.0665
OD ₅₇₀ measurement 2	0.7665	0.9482	0.9509
MLA-3202			
OD ₅₇₀ measurement 1	1.0080	0.7824	0.8794
OD ₅₇₀ measurement 2	1.0226	0.7907	0.7268
Positive control			
OD ₅₇₀ measurement 1	0.1570	0.1895	0.1112
OD ₅₇₀ measurement 2	0.1562	0.1653	0.1086

OD = Optical density

Triplicate exposures are indicated by A, B and C.

APPENDIX 3
Historical control data for *in vitro* skin irritation studies

	Negative control (absorption; OD ₅₇₀)	Positive control (absorption; OD ₅₇₀)	Positive control (viability; %)
Range	0.676 – 1.336	0.028 – 0.408	2.46 – 42.99
Mean	1.06	0.16	14.78
SD	0.14	0.11	9.29
n	115	115	115

SD = Standard deviation

n = Number of observations

The above mentioned historical control data range of the controls were obtained by collecting all data over the period of December 2012 to December 2015.

APPENDIX 4

Technical data, safety sheet and certificate of analysis reconstructed human epidermis



TECHNICAL DATA, SAFETY SHEET AND CERTIFICATE OF ANALYSIS RECONSTRUCTED HUMAN EPIDERMIS

CCE-091-SM D13-S/01

Description:	Episkin Small Model 0.38 cm ² reconstructed epidermis of normal human keratinocytes. Cells are grown on a collagen matrix, for 13 days.										
Usage:	FOR SCIENTIFIC USE ONLY - PRODUCT OF HUMAN ORIGIN										
Storage:	This product was prepared and packaged using aseptic techniques. Store in an incubator at 37°C, 5% CO ₂ with saturated humidity.										
Passage:	Second (Strains n° : 09-KERA-009, 11-KERA-002, 08-KERA-001, 10-KERA-005)										
Batch N°:	16-EKIN-019										
Origin:	Adult donors.										
Histology:	 Control n° E161125										
Quality Controls:	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center; padding: 2px;">Test</th> <th style="text-align: center; padding: 2px;">Specification</th> <th style="text-align: center; padding: 2px;">Result</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; padding: 2px;">Histology scoring (HES stained vertical paraffin sections, n = 6)</td> <td style="text-align: center; padding: 2px;">≥ 19.5</td> <td style="text-align: center; padding: 2px;">$22.3 \pm 0,3$ (CV = 1.2 %)</td> </tr> <tr> <td style="text-align: center; padding: 2px;">IC 50 determination (SDS concentration, MTT test, n = 14)</td> <td style="text-align: center; padding: 2px;">$1.5 \text{ mg/mL} \leq \text{IC50} \leq 3 \text{ mg/mL}$</td> <td style="text-align: center; padding: 2px;">2.7 mg/mL</td> </tr> </tbody> </table> <p>Statistical Analysis : → Histology : probability 0.95 that 100 % of the batch > 20 → IC 50 : probability 0.95 that IC 50 $\geq 2.6 \text{ mg/ml}$ (threshold value)</p>		Test	Specification	Result	Histology scoring (HES stained vertical paraffin sections, n = 6)	≥ 19.5	$22.3 \pm 0,3$ (CV = 1.2 %)	IC 50 determination (SDS concentration, MTT test, n = 14)	$1.5 \text{ mg/mL} \leq \text{IC50} \leq 3 \text{ mg/mL}$	2.7 mg/mL
Test	Specification	Result									
Histology scoring (HES stained vertical paraffin sections, n = 6)	≥ 19.5	$22.3 \pm 0,3$ (CV = 1.2 %)									
IC 50 determination (SDS concentration, MTT test, n = 14)	$1.5 \text{ mg/mL} \leq \text{IC50} \leq 3 \text{ mg/mL}$	2.7 mg/mL									
Biological safety:	On blood of the same donors, we have verified: . the absence of HIV1 and 2 antibodies . the absence of hepatitis C antibodies . the absence of hepatitis B antigen HBs On epidermal cells of the same donors, we have verified: . the absence of bacteria, fungus and mycoplasma										
Expiration date	May 16, 2016.										

"The use of this human tissue is strictly limited to in vitro testing. All other manipulations of this tissue such as: extraction and maintenance of single cells in culture, use of the tissue for diagnostic or therapeutic purposes and in human subjects, are strictly prohibited"

Lyon, May 10, 2016.

Certified and released by

Michel BATAILLON, Quality Control Manager

Manufactured in accordance to the ISO9001 quality system of Episkin.

ISO 9001 Certified

4, rue Alexander Fleming - 69366 Lyon Cedex 07 - France - Tél : +33 (0)4 37 28 72 00 - Fax : +33 (0)4 37 28 72 28
S.A. au capital de 13 608 807 € - 412 127 565 R.C.S. Lyon - NAF : 7211 Z - N° TVA Intracommunautaire FR 46 412 127 565
www.episkin.com



APPENDIX 5

Certificate of analysis



Chemtura Corporation
12 Spencer St
Naugatuck, CT 06770

Analytical Services
www.chemtura.com

Certificate of Purity

Customer: Support for Toxicology Studies

Test Substance Name: MLA3202; Amides, tallow, N,N-bis(2-hydroxypropyl)

Physical Appearance: Liquid

CAS No.: 1454803-04-3

Ref. or Lot Number: RC-1045

Date of Analysis: revised March 18, 2016 (original issue March 7, 2016)

Percent Composition	Monoisotopic Mass (daltons)	Formula	Structure/ Identity
33.1	397.4	C ₂₄ H ₄₇ NO ₃	C18:1 (oleic) tallow amides, N,N-bis(2-hydroxypropyl)
22.9	371.3	C ₂₂ H ₄₅ NO ₃	C16:0 (palmitic) tallow amides, N,N-bis(2-hydroxypropyl)
13.6	395.4	C ₂₄ H ₄₅ NO ₃	C18:2 (linoleic) tallow amides, N,N-bis(2-hydroxypropyl)
11.0	399.4	C ₂₄ H ₄₉ NO ₃	C18:0 (stearic) tallow amides, N,N-bis(2-hydroxypropyl)
6.0	369.3	C ₂₂ H ₄₃ NO ₃	C16:1 (palmitoleic) tallow amides, N,N-bis(2-hydroxypropyl)
3.2	419.3	C ₂₆ H ₄₅ NO ₃	C20:4 (eicosatetraenoic) tallow amides, N,N-bis (2-hydroxypropyl)
2.0	393.3	C ₂₄ H ₄₃ NO ₃	C18:3 (linolenic) tallow amides, N,N-bis(2-hydroxypropyl)
1.5	282.3	C ₁₈ H ₃₄ O ₂	C18:1 (oleic) acid
1.1	421.4	C ₂₆ H ₄₇ NO ₃	C20:3 (eicosatrienoic) tallow amides, N,N-bis (2-hydroxypropyl)
5.6			Sum of residual components (< 1% each)
100.0			Total

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